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Role of genetic aspect in pathogenesis of atopic dermatitis

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ABSTRACT

The pathogenesis of atopic dermatitis (AD) is a very complicated process that involves an intricate array of molecules. Nowadays it is generally accepted that cytokines play an important role in the progression of the clinical presentation of atopic dermatitis. However, emerging data point to the possible involvement of cornified envelope proteins in the development of skin barrier dysfunction and illness. Unfortunately, our knowledge on relation of particular genotype to progression of AD is very limited. Therefore, intensive studies are needed to increase our understanding of genetic background of atopic dermatitis. Hopefully the future research will identify new factors that help us to determine the additional risk for certain patients with atopic dermatitis.

KEY WORDS: allergic disease, SNPs, eczema, cornified envelope, interleukins

Introduction

Atopic dermatitis (AD, atopic eczema) is an inflammatory, chronic and recurrent dermatosis, whose dominant symptom is persistent and severe pruritus. Skin changes usually appear in early childhood, favoring typical locations and having a characteristic appearance (Leung & Bieber 2003). Living conditions and children maturation in the developed countries have fundamentally changed over the past years. The differences include frequency of infectious diseases, their treatment, contact with microorganisms, diet, chemical composition and pollution of air, all of which are connected with the constantly increasing incidence of allergies, especially in childhood and youth. The prevalence of atopic dermatitis has doubled or tripled in the industrialised countries over the past three decades; 15% to 30% of children and 2% to 10% of adults are currently affected by the illness (Williams & Flohr 2006). Atopic dermatitis is among the most frequently appearing skin diseases and is capable of coexisting with other IgE dependent atopic illnesses, e.g. with bronchial asthma, rash, allergic catarrh of the upper respiratory tract and nutritional allergy (Jansen *et al.* 1973). Type I (allergy) of hypersensitivity are the underlying reason for these illnesses. They represent a special kind of reaction of the organism. Sometimes a disproportionately small dose of antigen triggers dramatic manifestations (Custovic & Simpson 2012). The etiopathogenesis of AD is complex and still unexplained; immunologic, environmental and genetic factors are involved, and should be considered in the context of genes encoding structural and functional proteins of the epidermis and main elements of the immune system (Bieber 2008). The international HapMap project was started in 2002 to develop a public database that could help researchers find genes associated with human diseases and

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individual responses to pharmacological agents (http:hapmap.ncbi.nlm.nih.gov). Also, genome-wide association studies (GWAS) investigate the relationship between disease and common genetic variants spread across the genome (McCarthy *et al*. 2008). Meta-analyses have enabled researchers to distinguish loci of susceptibility to atopic dermatitis located on ten different chromosomes: 1, 2, 3, 5, 6, 7, 10, 11, 19, 20. In European populations, the loci 4q27, 5q31, 11p13, 11q13, 16p13.13, 17q21.32 and 19p13.2 were identified (Tamari *et al*. 2013; Ellinghaus *et al*. 2013). At present the attention of researchers is focused on seeking genes whose mutations or specific allelic forms predispose organisms to development of AD. Identification of genetic polymorphisms due to single nucleotide polymorphisms (SNPs) is the most common approach for finding genetic factors conditioning susceptibility to disease.

Genetic factors that influence development of the AD phenotype

Role of the skin barrier

Defects of the skin barrier, which physiologically constitutes the natural protection of the organism, are clearly associated with the disease phenotype (Boguniewicz & Leung 2011). The cornified envelope is the most important layer of epidermis due to the protection it from physical injuries and exogenous compounds. It consists of a number of dead, completely cornified cell layers. These cells are quite elastic, which helps them to fulfill their function. The cornified layer is hydrophobic thus, protects the skin against penetration of water from the environment and prevents dehydration of the organism (Alasdair *et al.* 1994). The cornified envelope consists of specialized proteins: loricrin (LOR), small proline-rich proteins (SPRR), a family of fused-type S100 proteins composed of filaggrin (FLG), repetin (RPTN), cornulin (CRNN), hornein (HRNR), and late cornified envelope-like proline-rich 1 (LELP1). Genes encoding these proteins are found within the epidermal differentiation complex (EDC), a gene cluster whose products are responsible for the final diversification of keratinocytes (Kypriotou *et al.* 2012). These proteins form a thick layer resistant to physical and chemical factors, influence production of natural moisturizing compound and ensure the appropriate pH of the skin, that prevents the penetration of infectious factors to its deeper layers. However, in AD patients, more rapid desquamation of the cornified layer occurs accompanied by exaggerated degradation of ceramides, which results in increased loss of water and increased permeability to exogenous allergens. This is frequently followed by the development of inflammation (Proksch *et al.* 2006). Moreover, reduced level of antimicrobial factors in the epidermis frequently results in recurrent bacterial infections in affected individuals (Bieber 2008). Therefore, genetic alterations including polymorphisms and mutations in genes encoding the proteins involved in the proper building of the epidermal barrier, may carry a certain degree of risk of the appearance of atopic dermatitis; this at present is being intensively studied.

Recent results of scientists from the University of Dundee provide a breakthrough in the area of AD genetics by revealing that *FLG* null alleles are a frequent transmissible predisposing factors in common atopic dermatitis. This study documented that inherited reduction or loss of filaggrin expression is a major predisposing factor in AD, and provided a molecular mechanism to define the coexistence of a clinical subtype of asthma (Palmer *et al*. 2006). These results initiated a flurry of research on this protein. Filaggrin functioning rely on binding of keratin fibers in the process of keratinocytes maturation. As a result of FLG conversion, a natural moisturizing factor is produced (Gan & McBride 1990). The metabolism of this protein result in an acidic pH generation in the cornified layer, an optimal environment for the enzymes synthesizing lipids of the cornified envelope (CE) (Markova *et al.* 1993).

R501X and 2282del4 are the most frequently occurring mutations of *FLG*. It was demonstrated that lack of FLG expression or its decrease in the skin caused by gene mutation occurs mainly in patients with early onset of the disease, a severe course of AD and elevated IgE levels (Palmer *et al*. 2006). It must be emphasized that *FLG* mutations occur only in a portion of AD patients. Moreover, in 9% of the European population, despite observed mutations, the atopic dermatitis phenotype was not developed. These results indicate the possible significance of polymorphisms and mutation within other than *FLG* genes encoding proteins of the epidermal differentiation complex (EDC) in the development of AD.

Loricrin is a major protein component of the cornified cell envelope found in terminally differentiated epidermal cells. It is a glycine-serine-cysteine-rich protein, synthesised in the granular (*stratum granulosum*) layer (Hohl *et al*. 1991). A connection between abnormal expression of *LOR* and skin diseases has been proven. Data has shown that mutation 730insG in *LOR*, which elongates loricrin by 22 amino acids due to delayed termination, is a factor in honeycomb palmoplantar keratoderma and the diffuse-ichthyosis form of dermatosis (Gedicke *et al.* 2005). Another study found that the down-regulation of loricrin and filaggrin was accompanied by up-regulation of some keratins in active AD skin lesions. The authors suggest that deterioration of epidermal differentiation associated with altered expression of genes located on 1q21 might be a key abnormality in atopic dermatitis (Weldinger *et al.* 2013).

The next gene to be identified as a possible factor in the development of AD is *LELP-1*. This gene encodes a late cornified envelope-like prolin-rich protein. Indian scientists found a significant link between rs7534334 SNP and log10 serum IgE levels in the group of patients (Sharma *et al*. 2007). However, this was only a single study and the authors stressed the need for further research.

A single nucleotide polymorphism within the gene encoding hornerin has recently been linked with susceptibility to atopic dermatitis (Henry *et al.* 2011). In the epidermis, hornerin was found to be co-localised with profilaggrin in keratohyalin granules in cells of the granular layer. These findings indicate that hornerin has a function similar to or mutually complementary to profilaggrin in the cornifying epithelium (Makino *et al.* 2001). Human protein hornerin was detected in regenerating skin following a wound and in psoriatic skin (Takaishi *et al.* 2012). It has been reported that allele C of rs877776 in *HRNR* gene is a risk factor of increased frequency of AD compared to controls even following exclusion of *FLG* mutation carriers (Esparza-Gordillo *et al.* 2009). In an Austrian population, single nucleotide polymorphisms, rs7927894 on chromosome 11q13.5 within the region of the *HRNR* gene, was identified as novel susceptibility variant for atopic dermatitis (Greisenegger *et al.* 2013). This study point to the statistically significant association of the rs7927894 variant with AD, but not with other disease-related phenotypes. Therefore, authors of that study postulated that the rs7927894 single nucleotide polymorphism selectively influences eczema development.

Repetin, a protein consisting of 784 amino acids, has a structure resembling the helix-calcium-binding-loop-helix domain of parvalbumin, hands of the S100 type and internal tandem repeats typical for CE precursor proteins (Huber *et al.* 2005). This protein associates with keratin intermediate filaments and is partially cross-linked to the cell envelope (Krieg *et al.* 1997). It has been proposed that this protein may be a marker of disturbances in differentiation of skin barrier cells and may be significant in the development of atopic dermatitis.

Polymorphism and mutations in the *CRNR* gene may also be associated with AD. Data has shown that human cornulin mRNA is expressed primarily in the upper layers of differentiated squamous tissues including the epidermis (Contzler *et al.* 2005). Data concerning eczema in Swedish families has shown that the *CRNN* polymorphism rs941934 is significantly associated with atopic eczema in the genetic analysis,

although only as part of an extended haplotype including a known associated variant 2282del4 in the filaggrin gene (Liedén *et al.* 2009).

The epidermal differentiation complex genes also encode the precursor protein of the cornified cell envelope, such as small proline-rich proteins (Hohl *et al.* 1995). The *SPRR* gene class consists of two *SPRR1* and seven *SPRR2* genes, along with a single *SPRR3* gene (Kartasova & van de Putte 1988). In human cornea tissue, the expression of SPRR1, SPRR2 and filaggrin protein were detected in the central and peripheral corneal and limbal epithelium (Tong *et al*. 2006). Cabral *et al.* noticed that the structural organization and regulation of the *SPRR* gene family reflects the epithelial barrier's role i.e. guarantee optimal protection to the organism (Cabral *et al.* 2001). Nomura *et al.* recently reported that SPRR2C, a component of the CE with a protective skin barrier function, showed the largest (eleven-fold) increase in psoriatic skin lesions as compared with AD (Nomura *et al.* 2003). Polish investigators noticed the deregulated increase in *SPRR* expression in chronic atopic skin lesions; *SPRR1A* and *SPRR2C* lose their coexpression with *S100* genes and other 1q21 transcripts (Jarzab *et al.* 2009). They hypothesize that this altered pattern reflects an insufficient rise in *SPRR* expression, which is unable to compensate for the lack of loricrin and thus contributes to the persistence of chronic AD skin changes.

The correlation of gene polymorphism with atopic disease was also observed. The data suggested a dominant mode of inheritance for the risk allele of *SPRR3* in eczema (Marenholz *et al.* 2011). In this study the frequency of appearance of the gene polymorphism rs28989168 among the AD patients and control group was analyzed. It appeared that the *SPRR3* variant associated with atopic dermatitis carried an extra 24-bp repeat in the central domain, which may alter the physical properties of the CE (Marenholz *et al.* 2011).

To sum up, among EDC genes several genes have been identified as factors contributing to the risk of AD development. This point the need of further research particularly since the present results have not been confirmed by independent laboratories and are mostly incomplete. More investigations involving distinct study populations are needed to assess the role of identified polymorphisms in atopic dermatitis. Identification of the genes that are deregulated in the atopic organism is thus likely to improve our understanding of AD pathogenesis [Tab.1].

Role of interleukins

Interleukins are molecules that regulate diverse processes, e.g. the proliferation, differentiation and mobility of cells. Acting on many cells, interleukins are mediators of inflammatory responses and immunologic processes. Also, keratinocytes, in response to barrier dysfunction, produce a variety of cytokines (Kayserova *et al.* 2012, Maeve *et al*. 2013). Therefore, investigators are also focused on examining genes encoding interleukins, which may play a role in development and progression of AD.

Interleukin 4 (IL-4) is produced through stimulation Th lymphocytes by an antigen. A correlation exists between IL-4 secretion and IgE concentration in plasma; its increased expression causes inflammatory responses of an allergic character (Namkung *et al*. 2011a). Czech investigators analyzed polymorphism in IL-4 receptor α (*IL-4Rα*) at position +1902 in patients with AD and a control group. This work showed a significant association between the genotypes of IL-4Rα and an increased level of tree-pollen-specific IgE (Kayserova *et al.* 2012).

The initial finding that interleukin-7 (IL-7) is produced by human keratinocytes suggested its possible involvement in skin diseases. A polymorphism T244I of receptor IL-7R was also found to increase the risk of AD in a group of German patients (Hoffjan *et al*. 2010).

Interleukin 9 (IL-9) is produced by stimulated T-lymphocytes, particulary Th2. This cytokine plays an important role in the regulation of antiparasitic response. It has

been suggests that SNP rs31563, located within the *IL-9* gene, is associated with increased susceptibility to AD (Namkung *et al*. 2011b). Similarly, rs3093467 SNP in the IL-*9R* gene seems to be associated with an increased risk of developing non-allergic AD (Namkung *et al*. 2011b).

Interleukin 12 and 13 (IL-12 and IL-13) are other cytokines that may play a critical role in AD. IL-12 is produced by antigen-presenting cells. It can also be secreted by keratinocytes (Namkung *et al*. 2010). IL-13 is similar in its action to IL-4, and receptors for these two cytokines share a common subunit (Hussein *et al*. 2011). Single nucleotide polymorphisms in IL-4 and IL-13 have been reported in patients with allergic diseases. Korean researchers noticed that two SNPs, rs3091307 and rs20541, from the *IL-13* gene showed a significant difference in allelic or genotypic distributions between AD and normal groups. However, they did not observe any associations for the *IL-4Rα* polymorphism C3223T or the *IL-4* polymorphism C590T (Namkung *et al*. 2011a).

TABLE 1. Genes with polymorphism and mutation linked to AD risk.

protein	**gene**	**SNP/mutation**	**Allel 1**	**Allel 2**	**Chromosome loci**	**Functional group**
Interleukin 9	*IL-9*	rs31563	C	T	5:135235606	cytokine-related
Interleukin 7 receptor alpha chain	*IL-7R*	rs11567705	C	G	5:35861152	cytokine - related
Interleukin 13	*IL-13*	rs3091307	A	G	5:131989136	cytokine - related
Interleukin 13	*IL-13*	rs20541	A	G	5:131995964	cytokine - related
Interleukin 18	*IL-18*	rs360721	C	G	11:112025916	cytokine - related
Filaggrin	*FLG*	R501X	C	T	1:152285861	skin barrier
Hornerin	*HRNR*	rs877776	C	G	1:152178018	skin barrier
Cornulin	*CRNR*	rs941934	C	T	1:152390452	skin barrier
Late cornified envelope-like proline-rich 1	*LELP-1*	rs7534334	C	T	1:153177852	skin barrier

Interleukin 10 (IL-10) fulfils many functions which result in suppression of immune response on a cellular level and inflammatory response (Lacy *et al*. 2009). Examinations conducted among children under the age of 3 years have shown the

potential role of *IL10* SNPs in the development of immune-mediated diseases, such as AD (Raedler *et al*. 2013).
Keratinocytes and epithelial cells can secrete interleukin 18 (IL-18). Genome – wide association study suggested IL-18R1 role for interleukins signaling (Hirota *et al*. 2012). Ibrahim *et al*. noticed that the -140 GG genotype and the -140 G allele were more often observed in patients with severe AD compared with mild and moderate phenotypes (Ibrahim *et al*. 2012).
Interleukin 31 (IL-31), produced by Th2 lymphocytes, acts on macrophages and keratinocytes. In these cells there is a receptor for this cytokine (Kasraie *et al*. 2013). It is postulated that IL-31 plays a role in AD pathogenesis. Associations between *IL-31* gene variants and eczema have previously been demonstrated in three independent European populations (Schulz *et al*. 2007).
The data accumulated to day indicate that the level of expression of genes encoding interleukins has a critical influence on the developing atopic dermatitis. Interleukins SNPs status of the organism can have great meaning if we want predict risk of allergy [Tab.1].

Conclusion

Atopic dermatitis is a multifactorial-disease. The data we presently have at our disposal show that there is no simple correlation associated with a single gene defect or with the occurrence of its determined allelic form and the risk of contracting the disease. It seems that the interaction of many genes plays a role in the progression of the disease. Moreover their expression is influenced by environmental factors. All authors emphasize the need to conduct intensive research to clarify the genetic basis of AD.

Determination whether a link exists between the frequency of appearance of the particular variant of the polymorphic gene coding protein s forming the cornified envelope or specific interleukins, on one hand, and the risk of atopic dermatitis on the other, will help us to better understand the pathogenesis of this disorder. This will influence the direction of research on new therapeutic methods and enable the development of a more effective and safer treatment for atopic dermatitis.

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Inhibitors of bacterial and plants urease. A review.

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ABSTRACT

Urease is an important virulence factor for *Helicobacter pylori* and *Proteus mirabilis* as well as in environmental transformations of certain nitrogenous compounds. Urea hydrolysis caused by these microorganisms leads to increased pH and ammonia toxicity and enables bacterial colonization of the human gastric mucosa and urinary tract formation of struvite and carbonate-apatite stones. Due to the possibility of medical applications the development of novel, selective and efficient classes of urease inhibitors which satisfy the low toxicity requirement for human health and have low environmental impact is necessary. In this article are described the various urease inhibitors used so far by researchers, especially in the last few years.

KEY WORDS: urea, urease, inhibitor, ulcer disease, kidney stones

Introduction

Urease (urea amidohydrolase EC3.5.1.5) is a large heteropolymeric enzyme which belongs to the super family of amidohydrolase (Sander *et al.* 1997). It catalyses the hydrolysis of urea to ammonia and carbamate. High concentration of ammonia arising from the reaction, as well as the accompanying pH elevation, has important negative effects in the fields of medicine and agriculture (Polacco *et al.* 1995). Many microorganisms utilize urea as a source of nitrogen for augmentation, for example: bacteria, fungi, algae. Urease plays a pivotal role in nitrogen metabolism of plant during the germination process (Polacco *et al.* 2002; Zhu *et al*. 2009).

The order of amino acids and their enzymatic mechanism in all the ureases is preserved. The X-ray crystallographic studies on bacterial ureases derived from *Sporosarcina pasteurii* (*Bacillus pasteurii*), *Klebsiella aerogenes* and *Helicobacter pylori* gave a detailed insight into the structure of its active site. These investigations have enabled to propose the general mechanism of urea hydrolysis (Ciurli *et al.* 2013; Mangani *et al.* 1996; Karplus *et al.* 1995). It was found, that inside the active center two nickel ions are held in separation bridged by carboxylate group of carbamylated Lys and hydroxide originated from the water molecule. Both ions are further coordinated by two histidines, while one of them forms extra bond with Asp. Binuclear metallocenter is additionally stabilized by hydroxide cluster that fills active site cavity of the native enzyme (Mangani *et al.* 1999).

Urease activity is widely distributed in soil and aquatic environments, where it plays a significant role in nitrogen metabolism (Burns 1978). In many environments the level of available nitrogen compounds is inadequate for optimal plant production. Therefore,

fertilizers are applied which can be converted to a form of nitrogen that plants can assimilate (Beaton 1978; Hausinger *et al.* 1989). High concentrations of ureases cause significant environmental and economic problems by releasing enormous amounts of ammonia into the atmosphere during urea fertilization (the most widely used fertilizer). It induces plant damage by depriving of their essential nutrients and secondly through ammonia toxicity and carbon dioxide release which increase the pH of the soil (Bremner 1995). Therefore effective methods are needed to solve the problems encountered in the use of urea as fertilizer (Balasubramanian & Ponnuraj 2010). One of the approaches is the inhibition of the urea hydrolysis. Urease inhibitors could have a practical value as the active additives to nitrogen fertilizers, that could regulate the excessive rates of ureolysis in soil (Schwedt *et al*. 1993).

Ureases are important enzymes in some human and animal pathogenic states, they are responsible for kidney stones entailed in urolithiasis that contributes toward the acute pyelonephritis with other urinary tract infection. Furthermore urease contributes to arthritis and gastric intestinal infections and ultimately the urease imbalance lead to peptic ulcers. This pathologies are caused by *Helicobacter pylori* and *Proteus mirabilis*. The obvious remedy for treating bacterial infection are antibiotics, however often proved to be unsuccessful (Polacco *et al.* 1995; Choudhary *et al.* 2008; Hausinger *et al.* 1995; Hausinger *et al.* 1989).

Strategies based on urease inhibition are the main treatment of diseases caused by urease producing bacteria. Enzyme inhibition is an important area of pharmaceutical research. Studies in this field have already led to the discovery of wide variety of drugs useful in a number of diseases and have been used for treating a number of physiological conditions. Specific inhibitors interact with enzymes and block their activity towards their corresponding natural and synthetic substrates (Choudhary *et al.* 2002).

The standard of urease inhibitors

Urease inhibitors belong to different chemical classes and many of them have been investigated in the past decades, for example hydroxamic acids, which was found to be effective against a wide range of bacterial ureases and is the best recognized urease inhibitors (Polacco *et al*. 1995; Uehare *et al.* 1962; Williamson *et al*. 2003). Phosphoramidates are the most potent compounds, which have found applications in agriculture as soil urease inhibitors (Choudhary *et al.* 2002; Orlinska *et al.* 2001). Hydroxamic acids and phosphoroamide compounds create tetrahedral intermediate with a structural similarity to the tetrahedral intermediate postulated to occur during urea hydrolysis. However, because of teratogenicity of hydroxamic acid in rats (Schmidt *et al*. 1968) and degradation of phosphoramidates at low pH (Garcia-Mina *et al.* 2008)prevented their use as a drug in vivo. Boric and boronic acids are suggested to form a complex with nickel ion(s) (Breitenbach & Hausinger 1988). Quinone derivatives are the another class of compounds that showed enzyme inhibitory activities. Heavy metal ions interact with thiolgroups of cysteine residues. The reaction is analogous to the formation of metal sulphide (Krajewska 1991). A promising group of urease inhibitors constitute polyphenols, widely used as biologically active food additives due to their high antioxidant properties. The example is gallocatechin, a polyphenol which is extracted from green tea, naturally occurring flavonoids have been reported as inhibitors of *Helicobacter pylori* urease (Sugimura *et al.* 2003; Kim *et al.* 2005). Thiols are not potent inhibitors however the presence of other charged groups has a significant effect on the inhibition constant (Todd & Hausinger 1989). Ureases have long been known for their sensitivity to the inhibition by heavy metal ions. The inhibition of ureases by bismuth compounds has been mainly tested for their potential application in the treatment of peptic

ulcers and Helicobacter pylori infections, because bismuth compounds are widely used as bactericidal agents (Zhang *et al.* 2006; Krajewska 2009). The relative effectiveness of the heavy metal ions as inhibitors of jack been urease has been reported to decrease in the following approximate order: $Hg^{2+}>Ag^{+}>Cu^{2+}>Ni^{2+}>Cd^{2+}>Zn^{2+}>Co^{2+}>Fe^{3+}>Pb^{2+}>Mn^{2+}$(Krajewska 2008).

These are only examples of urease inhibitors. The part of them cannot be used in vivo because of their toxicity or instability, therefore the development of novel classes of selective and efficient urease inhibitors which satisfy the low toxicity requirement for human health and have low environmental impact is necessary. In the last few years, many potent inhibitors have been obtained and reported in the literature.

The new class of urease inhibitors

Recently, a series of new and novel Schiff base derivatives showed significant inhibitory activity against Jack bean urease (Zhu *et al*. 2007) due to the similarity of their basic skeleton with urease substrate. The most potent inhibitors werecompounds with K_i=0.09 μM and K_i=0.122 μM (Figure 1, compound 1 and 2). All of the compounds showed competitive mechanism of inhibition (Iqbala *et al.* 2011). Schiff base hydrazones are well known class of compounds, possess various activities like antimicrobial (Blandini *et al.* 1995), antimycobacterial (Kandefer-Szerszen *et al.* 2007), antitumor, anti-inflammatory (Soares *et al.* 2006), antimalarial (Wang *et al*. 2006) and antidiabetic activities (Sharma *et al.* 2012). Scaffold of Schiff base urease inhibitors can be utilized in further optimization to improve potency and selectivity by variations in the basic skeleton.

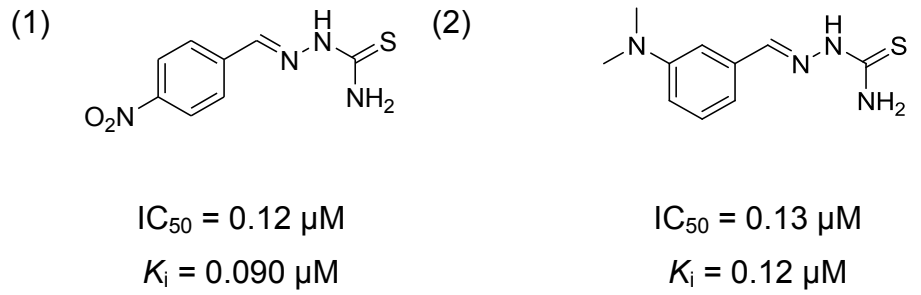

Figure 1. The structure of Jack bean urease inhibitors.

It is very interesting that, the study of urease inhibitors from natural products has attracted a lot of attention (Choudhary *et al*. 2012; Lateef *et al*. 2012). It is well known that structural diversity and complexity within natural products are unique and the functional complexity found in natural products is difficult to invented de novo in the laboratory (Häbich *et al*. 2006). In 2001, Bae *et al*. found flavonoids having weak inhibitory activity against *Helicobacter pylori* urease (Zhu *et al.* 2011). Based on these studies, Zhu-Ping Xiao and his co-workers synthesized and evaluated nineteen derivatives of flavonoids against *Helicobacter pylori* urease. Analysis of structure activity relationship disclosed that 4-deoxy analogues are more potent than other products. Out of them, 4',7,8-trihydroxyl-2-isoflavene (Figure 2) was found to be the most active with IC_{50} of 0.85 μM, being over 20-fold more potent than the commercial available urease inhibitor, acetohydroxamic acid (Hai-Liang *et al.* 2013; Janser *et al.* 2013).

In 2003, Kawase *et al*. reported for the first time that several α,β-unsaturated ketones are

inhibitors for jack bean urease. The most potent compounds were cyclic and of low-molecular weight, e.g. 2-cyloheptene-1-one (IC_{50} = 0.16 mM), 2-cyclohexene-1-one (IC_{50} = 0.69 mM), 2-cyclopentene-1-one (IC_{50} = 0.97 mM) (Tani *et al*. 2003). The result of studies conducted by Kawase *et al*. in 2003 and then by Ingo Janser and his co-workers demonstrated that ethacrynic acid is potent inhibitory activity against jack bean urease, even at low concentrations (Tani *et al.* 2003; Krajewska 2009). Ethacrynic acid and a series of its analogues were synthesized and subsequently evaluated for their inhibitory effect on urease. The highest inhibitory activity was found for compound (5) (IC_{50} = 0.05 mM), compound (6) (IC50 = 0.07 mM), compound (4) (IC_{50} = 0.08 mM), and compound (7) (IC_{50} = 0.10 mM) (Figure 3). It is noteworthy that all four compounds possess a methoxy group at the aromatic system. They demonstrated that the α,β-unsaturated carbonyl unit of this compounds is mandatory to inhibit the enzyme. These studies require further follow-up (Janser *et al*. 2013).

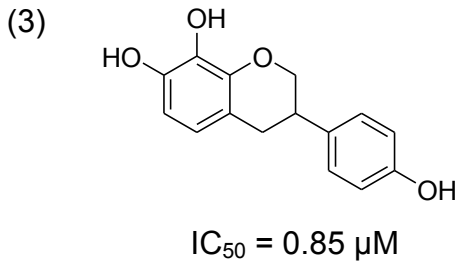

Figure 2. The structure of 4',7,8-trihydroxyl-2-isoflavene.

Of all the above mentioned classes of compounds, amides and phosphoric acid esters represents the group of the most exploited inhibitors towards both bacterial and plant ureases (Krajewska 2009). Several phosphorodiamidates and their thiophosphoric analogues were successfully introduced to agriculture to control hydrolysis of urea in soil and diminish nitrogen loss. Unfortunately, their possible therapeutical use is limited by low hydrolytic stability of P-N bond in acidic pH (Marzadori *et al*. 2009; Garcia-Mina *et al*. 2011; Berlicki *et al*. 2008).

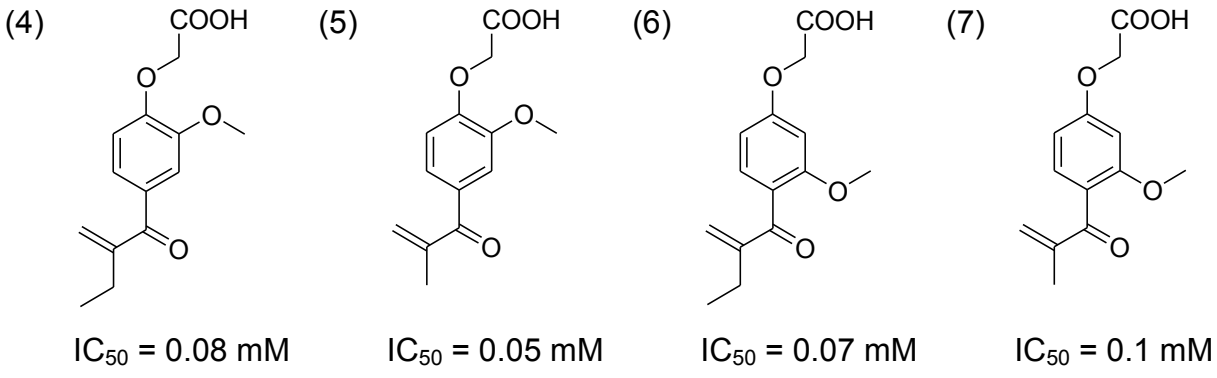

Figure 3. The analogues of ethacrynic acid.

Inhibitory properties of phosphinic and phosphonic acid derivatives towards urease for a long time are synthesized and analyzed in Wroclaw University of Technology in the Bioorganic Chemistry group (Figure 4).The idea of using this compound as urease

inhibitor corresponds to its structural similarity to the transition state of urea hydrolysis as well as to phosphorodiamidate, which is one of the most potent urease inhibitor. The research is also based on the assumption that, in comparison to hydrolytically unstable phosphorodiamidic acid, the phosphinic acid and its derivatives remained stable even at acidic pH due to the presence of highly inert P-C linkages (Berlicki *et al*. 2008; Berlicki *et al*. 2010; Kosikowska & Berlicki 2012).

The computer aided design using crystal structures of *Sporosarcinia pasteurii* urease allowed the development of the novel and potent inhibitors, *P*-methyl phosphinic acids, which example is the most active *N*-(N′-benzyloxycarbonylglycyl)aminomethyl(*P*-methyl)phosphinothioic acid with K_i= 170 nM and 45 nM against *Sporosarcinia pasteurii* and *Proteus vulgaris* enzyme, respectively (compound 8) (Berlicki *et al*. 2008). Introduction of *P*-hydroxymethyl group into the molecule resulted in considerable increase of the inhibitory activity against urease isolated from *Sporosarcinia pasteurii* and *Proteus vulgaris* as compared with their *P*-methyl counterparts obtained previously. The most potent inhibitors in this group of compounds is *N*-methylaminomethyl-*P*-hydroxymethylphosphinic acid with K_i=430 nM and K_i = 360 nM against *Sporosarcinia pasteurii* and *Proteus vulgaris* urease, respectively (compound 9) (Berlicki *et al*. 2010). In order to improve affinity of inhibitor structure to selected bacterial ureases were explored the potential of aminomethylphosphonic and *P*-methyl-aminomethylphosphinic acids as novel inhibitors. The *N*,*N*-dimethyl derivative both mentioned structures were the most effective withK_i=13 ± 0.8 µM and 0.62 ± 0.09 µM, respectively (compounds 10 and 11). This structures offer the possibility of various modifications, which might provide improved physicochemical and inhibitory properties (Kosikowska & Berlicki 2012).

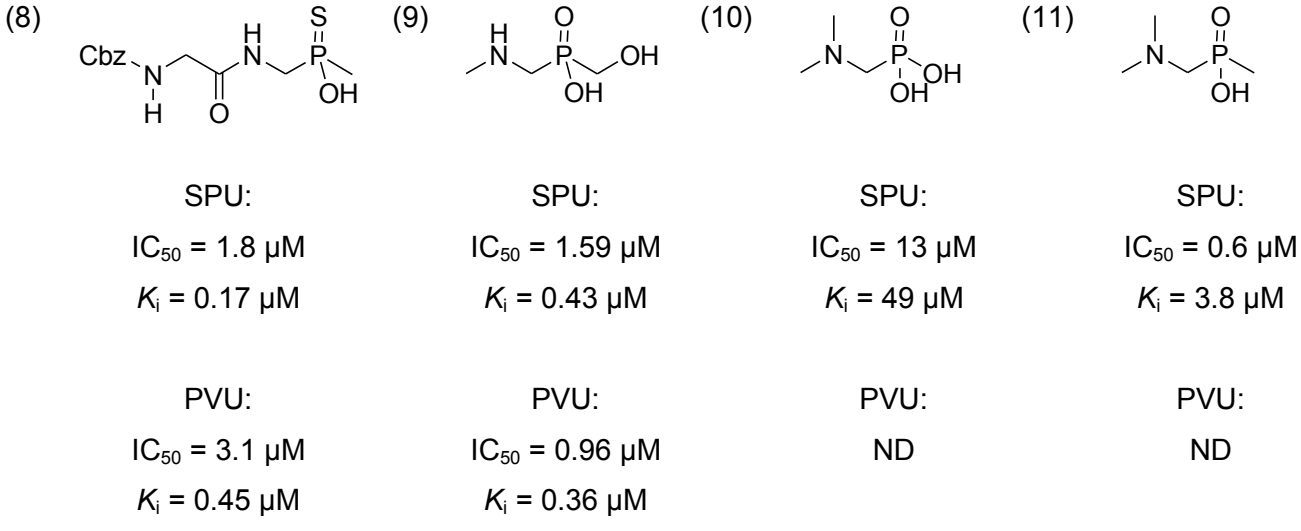

Figure 4. The structure of phosphinic and phosphonic urease inhibitors (SPU - Sporosarcinia pasteurii urease, PVU - Proteus vulgaris urease, ND - not determined).

Conclusions

Catalyzed hydrolysis of urea plays an important role as virulence factor for the urinary tract infections and gastrointestinal infections. Urease inhibition has become a growing area of research at the interface of the biomedical sciences, such as biology, chemistry, biophysics and biotechnology. A lot of potent inhibitors has been reported in literature. All the above mentioned research led to the synthesis of structures with low

structural complexity, high hydrolytic stability and satisfactory biological activity against bacterial and plants urease (*Proteus vulgaris* and *Proteus mirabilis*, *Sporosarcinia pasteurii*, *Helicobacter pylori*). Urease inhibitors are expected to bring interesting discovery in pharmaceutical, agricultural and environmental fields.

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Citrullination – small change with a great consequence

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ABSTRACT

Citrullination is one of the possible post-translational modifications of proteins. It is based on a conversion of L-arginine residue (L-Arg) to L-citrulline residue (L-Cit). The reaction is catalyzed by peptidylarginine deiminases (PAD). The change of L-Arg imino moiety results in a loss of a positive charge. This slight modification can contribute to significant changes in physicochemical properties of proteins, which may also cause a change of their functions. Citrullination is the modification observed in physiological processes such as epidermal keratinization, regulation of gene expression and the reorganization of myelin sheaths. The changes in the efficacy of citrullination may contribute to the pathogenesis of many different diseases including: psoriasis, multiple sclerosis, rheumatoid arthritis and cancer.

KEY WORDS: deimination, peptidylarginine deiminase, citrulline, post-translational modification

List of abbreviations: CARM1 - coactivator-associated arginine methyltransferase 1, **L-Arg-** L-Arginine, **L-Cit -** L-Citrulline, **MAGEA12 -** melanoma-associated antigen 12, **MBP -** myelin basic protein, **p21 -** CDK-interacting protein 1, **PAD -** peptidylarginine deiminase, **PRMT1 - N**-methyltransferase 1, **PRMT5 -** N-methyltransferase 5, **PTN -** pleiothropin, **RA -** rheumatoid arthritis, **TFF1 -** estrogen-responsive trefoil factor 1, **THH -** trichohalin

Citrulline and the reaction of citrullination

L-citrulline (L-Cit) is a ornithine derivative which is related to arginine (Fig. 1). It is found in nature in two forms: as a free amino acid or as an amino acid constituent of proteins.

Three enzymes are involved in the metabolism of free form of citrulline. Two of them originate from the urea cycle: ornithine carbamoyltransferase, which produces L-Cit, and argininosuccinate synthetase which converts L-Cit into argininosuccinate. The third enzyme, NO synthase, produces L-Cit as a by-product in NO production (Curis *et al*. 2005). Due to the importance of the L-Cit in the protein structure, its formation and function will be consider in details. Cit is not encoded by a t-RNA. The only way in which L-Cit can be introduced into proteins is the posttranslational modification of L-Arg - called citrullination. During the reaction, the easily protonated guanidine group of L-Arg is modified into an uncharged carbonyl group (Vossenaar *et al*. 2003). The most important results of this modification are: an altered isoelectric point of the protein, an alteration in the charge distribution and hydrogen or ionic bonds formation in the protein structure. The extensive citrullination of a protein may alter its tertiary structure, interaction with other molecules, cleavage regions, and its

solubility. The advanced degree of citrullination leads to denaturation of the protein (Tarcsa *et al*. 1996a; Vossenaar *et al*. 2003; Chang *et al*. 2005; Nakayama-Hamada *et al*. 2008).

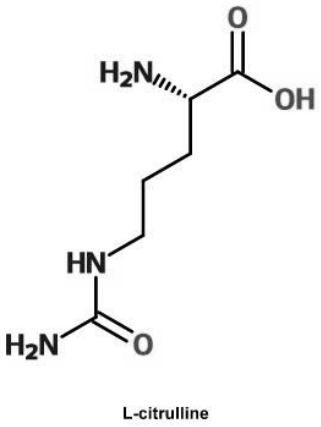

Figure 1. L-citrulline.

Mechanism of reaction

Citrullination is catalyzed by the action of Ca^{2+}-dependent enzymes belonging to the peptidylarginine deiminase family (EC 3.5.3.15). To date, five mammalian PAD genes have been identified. They are localized within the 334,7kb region in cluster 1p36.1. Because of high nucleotide sequence homology (59-71% identity) (Chavanas *et al*. 2006), and the conservative cluster organization it is postulated that PAD are the result of genetic duplications occurring before the divergence of mammalian species. The localization of enzymes and their mRNA with corresponding tissues is shown in Table 1 (Vossenaar *et al*. 2003; Suzuki *et al*. 2007).

As mentioned above, the target of the enzyme is the guanidine group of L-Arg (Fig. 2). Together with the catalytically important cysteine in PAD, it forms an intermediate tetrahedral adduct. Following a nucleophile attack of water molecules, the ammonia molecule is detached. Finally the ketone group is formed (Arita *et al*. 2004). Mammalian PADs have only the ability to convert proteinous L-Arg (or L-methyl-Arg) to L-Cit and free L-Arg is not modified by them (Takahara *et al*. 1986). This possibility possesses only peptidylarginine deiminase from *Porpyromonas gingivalis*, but the enzyme is not evolutionary associated with mammalian isoforms and its catalysis is independent of calcium ions (Rodríguez *et al*. 2010).

Conditions of reaction

The mammalian PAD action is dependent on calcium ions. Under physiological conditions, the Ca^{2+} concentration in the cell is 0,0001 mM, and it is too low to activate the enzymes. For example, PAD2 needs about 100-time higher ion concentration and half of its activity is obtained with 40 – 60 mM Ca^{2+} (Takahara *et al*. 1986; Vossenaar 2004). Because of this limitation, there are several situations in which activation of PAD is possible.

The first possibility concerns extreme conditions such as apoptosis (Asaga *et al*. 1998) or epidermis keratinization (Ying *et al*. 2012). The cell disintegration allows either the calcium ions influx, or enzyme exflux (Vossenaar 2004) to the intercellular space where the ion concentration is appropriate for activation. Alternatively, reservoirs of Ca^{2+} can be released from the mitochondria and endoplasmic reticulum, as is observed in prion disease (O'Donovan *et al*. 2001; Ferreiro *et al*. 2006; Jang *et al*. 2008).

Peptidylarginine deiminase reveals some preferences on the primary and secondary structure of the substrate. For example: Arg located close to Pro is never citrullinated and Arg situated in alpha helix is hardly deiminated. However, N-Arg-Asp-C is the one of most susceptible region for modification, like beta turns (György *et al*. 2006).

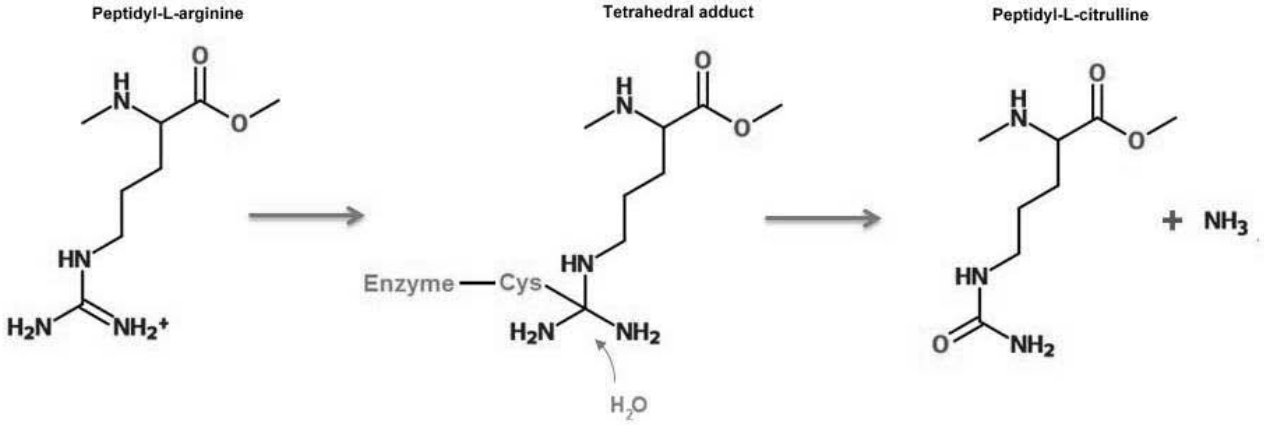

Figure 2. Mechanism of citrullination.

Citrullination in physiology and disease

Citrullination is a process which can occur in physiological or pathological conditions. In physiological processes, deimination play a regulatory role of processes such as the epidermal keratinization (Senshu *et al*. 1996), regulation of gene expression (Karlić *et al*. 2010) and myelin reorganization (Moscarello *et al*. 2002). In the pathogenesis of many diseases the reduction of appropriate level of protein citrullination was observed (psoriasis). On the other hand, the increased levels of L-Arg citrullination in physiological substrates (multiple sclerosis, progression of cancer) and modification of proteins that are not a physiological substrates for PAD was also presented (rheumatoid arthritis)(Vossenaar *et al*. 2003; Chang *et al*. 2011; Takizawa *et al*. 2006).

Epidermal keratinization

The skin is mechanical barrier providing protection against pathogens, skin-penetrating substances, and uncontrolled water loss. A lot of evidences point out the significant role of the protein citrullination in maintaining of the homeostasis and regulation of the keratinization process of the epidermis (Ying *et al*. 2012).

Keratinocytes migrate from the basal to the outer part of the epithelium, there they gradually die forming the stratum corneum. During cell differentiation, the calcium ions influx allows the activation of the three deiminase isoforms: PAD1, PAD2 and PAD3 (Vossenaar *et al*. 2003). Their main substrates include: keratin K1, keratin K10, and filaggrin (Nachat *et al*. 2005; Senshu *et al*. 1996).

Keratin K1 and K10 are fibrillar proteins expressed in the spinous and granular layers (Staquet *et al*. 1987). A deamination within keratin K1 concerns two preferred Arg residues located at Gly-rich subdomains V1 and V2. Within them are located the association sites for loricrin and desmosomes proteins such as desmoplakin (Senshu *et al*. 1999; Steinert *et al*. 1991). The change in the isoelectric point of cytokeratin as the results of citrullination improves its ability to interact with loricrin (Ishida-Yamamoto *et al*. 2000). It is worth mentioning here that due to a lack of Arg, loricrin is not subjected to modification.

Table 1. Expression of peptidylarginine deiminases in human tissues.

Enzymes		Expression sites	
	mRNA length (nts)	mRNA	proteins
PAD1	3846	colon, brain,ES cel, eye, inner ear, placenta, kidney, muscle, thymus, skin	uterus, epidermis
PAD2	4343	Brain, bone marrow, breast, colon, lung, muscle, skin, ovary, synovial membrane, synovial fluid	brain, salivary grand, uterus, macrophage, spleen, bone marrow, skin, synovial fluid, synovial membranę
PAD3	3183	skin, muscle, thymus	hair follicle
PAD4	2263	Brain, eye,fetal liver, bone marrow,kidney, spleen, leukocyte, synovial membranę, synovial fluid	Bone marrow, synovial membrane, synovial fluid, eosinophils, granulocyte
PAD6	2502	Embro, ovary (egg),thymus	Egg, ovary, early embro

The loss of basic character by K1 also affects the interaction with filaggrin - an important protein in the maintenance of epidermal homeostasis. During transition of keratinocyte to corneocyte, calpain 1 releases filaggrin monomers. These monomers are able to interact with keratin. This association results in the formation of an intracorneocyte fibrous matrix (Pearton *et al*. 2002). Some data suggest that citrullination of the filaggrin is crucial to its ability to dissociation and production of Natural Moisturizing Factor (amino acids constitute 52% of the composition)(Tarcsa *et al*. 1996b; Harding & Scott 1983; Ishida-Yamamoto *et al*. 2000).

Cytokeratin K1 and K10 are also linked to trichohalin (THH). It is an essential structural protein responsible for the mechanical strength of the hair follicle inner root sheath (Tarcsa *et al*. 1997). After synthesis THH form insoluble structures which are stabilized by ionic interactions between the alpha helices (Lee *et al*. 1993). During citrullination by PAD3, THH aggregates loosen their structure, making them more susceptible to crosslinking catalyzed by transglutaminase. THH complexed with both keratins leads to the formation of structures that are stable and insoluble in water (Tarcsa *et al*. 1997).

Disturbances in the citrullination are observed in skin diseases such as psoriasis. The disease is characterized by excessive activity of skin cell divisions and defective cornification. Pathomechanism of psoriasis is not fully understood. It is known that cytokeratin K1 has reduced number of citrullinated L-Arg (Ishida-Yamamoto *et al*. 2000).

Regulation of gene expression

Regulation of gene expression takes place on many levels. One of them is the control of transcription via modification of histones (Karlić *et al*. 2010). Histones are basic proteins which provide a framework for organizing the genetic material in a higher-order structure. One of the possible post-translational modification acting as a regulatory process is citrullination. PAD4 is able to modify N-terminal part of the histone H2A, H3, H4 (Hagiwara *et al*. 2005; Mastronardi *et al*. 2006). PAD2 can only modify histone H3 (Cherrington *et al*. 2010).

PAD competes for the L-Arg with methyltransferase, which catalyzes the L-Arg methylation. Addition of methyl groups

results in sequential formation of mono-, and then dimethylderivative of an L-Arg. An asymmetric dimethylarginine (both methyl groups on one terminal nitrogen) is created by coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine N-methyltransferase 1 (PRMT1). This modification leads to the activation of gene expression. However, symmetrical dimethylation which is the result of protein arginine N-methyltransferase 5 (PRMT5) activity, inhibits transcription. PAD4 can citrullinate L-Arg, and its monomethylated form. Deimination will decondense chromatin and prevent the creation of dimethylated L-Arg derivative. It is worth mentioning that dimethyl L-Arg is not a substrate for PAD (Thompson & Fast 2006; György *et al*. 2006).

Citrullination contributes to the change in affinity of the transcription apparatus influencing the gene expression *(Wysocka et al*. 2006). Citrullination of histones by PAD4 correlated for example with repression of estrogen-responsive trefoil factor 1 (TFF1) gene and apoptosis-associated CDK-interacting protein 1 (p21) and OKL38 genes. PAD2 is involved in the regulation of pleiothropin (PTN) and melanoma-associated antigen 12 (MAGEA12) genes (Cherrington *et al*. 2012).

PAD overexpression and changes in their subcellular localization is often accompanied by certain types of cancer (Mohanan *et al*. 2012). Increased levels of PAD4 and its activity is presented in invasive carcinomas like lung adenocarcinomas, esophageal carcinomas with squamous differentiation, colorectal adenocarcinomas and bladder uterine carcinomas etc. (Wang *et al*. 2010). The progression of cancer is also related to the change of PAD2 localization. In the normal breast tissues PAD2 is localized in both the cytoplasm and the nucleus. Changing the location of nuclear PAD2 in certain types of cancer may cause changes in gene expression and cause malignant transformation (Mohanan *et al*. 2012).

Creation and reorganization of myelin sheaths

A myelin sheath is formed by oligodendrocytes in the central nervous system, and Schwann cells in the peripheral nervous system. The main purpose of the myelin sheath is electrically isolation of axon and an increase in the speed of propagation of an electrical impulse along the myelinated fiber (Kursula 2008).

Myelin sheaths are composed of proteins, like myelin basic protein (MBP) and lipid components. The protein molecules are basic. They interact with negatively charged lipids like gangliosides and phosphatidylserine. Lipid-protein interaction is a key element in the sheath formation (Boggs *et al*. 1999). Native MBP are able to form a tight, compact structure. Such a structure is not conducive to its reorganization. The mutual interaction of molecules may be affected by frequent post-translational modifications within the MBP including citrullination, deamidation and methylation. The change in the isoelectric point of the protein after modification significantly influences the sheaths relaxation (Beniac *et al*. 2000).

The enzyme involved in the citrullination of MBP is PAD2. The highest PAD2 expression is observed in the gray matter and the hypothalamus (Kubilus & Baden 1983). It is known that the amount of deiminated MBP changes dramatically during life. In children under 2 years old, almost all MBP are modified. The degree of citrullination correlates with the observed brain plasticity (Moscarello *et al*. 1994). The number of modified proteins decreases with age. In the adult brain, the amount of citrullinated MBP remains constant and represents about 20% of the total pool of MBP (Moscarello *et al*. 2002).

Hyper-citrullination of proteins is observed in various neurodegenerative diseases such as multiple sclerosis. Modification of MBP applies not only to the percentage of modified proteins (increase from 20% to 45%), but also to the amount of citrullinated L-Arg (increase from 6 to 18 residues) (Moscarello *et al*.

1994; Wood *et al*. 1996). It is noted that excessive citrullination may occur during a reduced methylation as a result of a lowered methyltransferase activity (György *et al*. 2006).

Hyper-citrullination of MBP contributes to the development of the autoimmune response. Modified MBP are more susceptible to degradation of cathepsin D. PAD2 is localized in CNS myelin and presents elevated activity in multiple sclerosis (MS) (Berlet 1987). The reaction is approximately 35x faster in comparison to native MBP (Cao *et al*. 1999). Peptide released by cathepsin D contains an immunodominant epitope (Pritzker *et al*. 2000). Immune cells such as lymphocytes infiltrate into nerve tissue cause: oxidative stress, local inflammation and myelin sheath destruction underlying demyelinating disease (Whitaker *et al*. 1980).

Blood clot formation

The clot is a structure formed by the components of the blood to stop bleeding and repair the damaged blood vessel. One of the main proteins involved in the blood coagulation cascade is fibrinogen. Structurally, this is a dimeric glycoprotein. The release of fibrinopeptides A and B from fibrinogen, catalyzed by thrombin, results in the formation of monomers with exposed polymerization sites. Monomers organize themselves spontaneously and form a labile and then cross-linked stable fibrin. Finally, red cells and platelets adhere to resulting structure and form a clot (Blombäck *et al*. 1978; Furie & Furie 1988; Nakayama-Hamada *et al*. 2008).

Extravascular clot formation usually accompanies inflammatory processes for instance rheumatoid arthritis (RA). RA is a systemic autoimmune disease characterized by inflammation of peripheral joint which leads to cartilage destruction and joint dysfunction (Firestein 2003). During the infiltration of inflammatory synovium, monocytes differentiate into macrophages and subsequently become activated. Sustained activation makes them susceptible to programmed death. During the macrophage apoptosis, PAD2 and PAD4 are activated and then leaked into the synovium *(Rodenburg et al.* 2000; Vossenaar 2004). Damaged cell products stimulate the retraction of endothelial cells, which facilitates the extravasation of fibrinogen and other plasma components (Méchin *et al*. 2007). Citrullination can indeed occur within the rheumatoid synovial tissue with many different L-Arg residues citrullinated in different proteins including fibrinogen (Okumura *et al*. 2009; Vossenaar 2004).

Cleavage sites for thrombin are located on the N-terminus of Aα and Bβ chains of fibrinogen. It falls between Arg^{16}-Gly^{17} in the Aα chain and Arg^{14}-Gly^{15} in Bβ chain. Deimination blocks the releasing of fibrinopeptides because of L-Arg modification and thus prevents the polymerization of fibrin. Citrullinated fibrinogen in this case acts as an uncompetitive inhibitor of thrombin reaction (Nakayama-Hamada *et al*. 2008). In addition, certain fibrinogen molecules can be converted to fibrin before deimination. It is perhaps possible due to the increased level of thrombin in the synovial fluid (So *et al*. 2003)

After fibrin citrullination, a reduction ability to degradation it by plasmin can also be observed. This serine proteinase cleaves the peptide bond near basic amino acids such as Lys and Arg. Deimination reduces the number of potential degradation sites (Sebbag *et al*. 2004).

Currently, it is unknown whether the formation of fibrinogen deposits is a primary or secondary cause of the disease. However, it is known that fibrinogen can stimulate an immune response in two ways; directly across immunogenic citrullinated alpha and beta chains (Masson-Bessière *et al*. 2001) and indirectly by stimulating production of IL-1, IL-8, IL-13 and TNF-alpha by macrophages, resulting in extravasation of fibrinogen and a cyclic process (Rubin & Sønderstrup 2004).

Conclusion

Citrullination has been observed in many physiological and pathological processes. Modification can drastically change the properties and thus the function of the protein. However, we should note, that the citrullination have mainly regulatory functions and its role in pathogenic processes is possible only under certain conditions.

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The influence of camouflage and prey type on predatory decisions of jumping spider

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ABSTRACT

Decisions made by predators during predatory encounters are often based on multiple factors that may influence the outcome of the encounters. For stalking predators their visibility to the prey and the ability of their prey to escape may be important factors influencing predatory success. Hence they are likely to adapt their predatory behavior when approaching prey on backgrounds with different camouflaging properties, but only if the prey is able to escape. To test whether jumping spiders flexibly adapt their predatory behavior to camouflaging properties of the background and prey type, the behavior of *Yllenus arenarius* (Araneae, Salticide), a cryptically colored jumping spider hunting leafhoppers (high escape potential) and caterpillars (low escape potential) on two types of background: matching and non-matching for the spiders was analyzed. Background color had a significant effect on the spiders' jumping distance and their predatory success, but only if the prey had a high escape potential. No differences occurred between backgrounds if the prey could not escape. On camouflaging background the spiders attacked leafhoppers from a shorter distance and had a higher success than on non-camouflaging background.

KEY WORDS: crypsis, predatory behavior, behavioral plasticity, salticid spider, *Yllenus arenarius*

Introduction

In recent decades it has become increasingly apparent that predatory behavior of jumping spiders is complex and flexible (Jakob *et al*. 2011; Nelson & Jackson 2011). Decisions the spiders make during predatory encounters are often based on multiple factors that may influence the outcome of the encounter. Jumping spiders have been reported to adapt their predatory behavior to various properties of their prey, such as the potential of the prey to escape (Edwards & Jackson 1993; Bear & Hasson 1997; Bartos 2007), the ability of the prey to detect the spider (Bear & Hasson 1997; Li *et al*. 2003) or to injure the spider (Li *et al*. 1999; Jackson & Carter 2001). Such dangerous prey can be approached differently when it is capable of attacking the spider or when its ability to defend itself is impaired (Li & Jackson 2003). Some communal jumping spiders of the genus *Portia* make especially intricate predatory decisions based on the presence or absence of their prey nest, the identity of spiders inside and outside the nest and the position of these spiders relative to each other at the nest (Jackson & Nelson 2012). Making decisions requires from the spiders visual assessment of their environment and visual prey identification, often from a distance, and jumping spiders, due to their unique eyes, possess such abilities.

Jumping spiders are typical day hunters with well developed eyes (Land 1969a, b). They have four pairs of simple eyes (Forster 1982). Three pairs of these eyes are relatively small 'secondary eyes' and function primarily as movement detectors (Land 1972, 1985), but may also be used in depth perception and initial categorization of moving objects (Zurek & Nelson 2012). One pair of large forward-facing 'principal eyes' is positioned at the front of the cephalothorax. Principal eyes possess a unique structure (Land 1969a, b; Blest *et al*. 1990) and provide spatial acuity unparalleled among any terrestrial invertebrates (Williams & McIntyre 1980; Harland & Jackson 2004). Some of the spiders can discriminate between objects spaced 0.12 mm apart from a distance of about 200 mm (Harland & Jackson 2004), which enables them to identify their prey based on a high degree of detail (Jackson & Nelson 2012; Nelson & Jackson 2012a, b). Jumping spiders can discern green, blue and ultraviolet (Land 1969a; Yamashita & Tateda 1976; Peaslee & Wilson 1989; Blest *et al*. 1981) and were reported to discriminate between differently colored backgrounds (Nakamura & Yamashita 2000).

Jumping spiders are stalking predators. They do not build prey-capture webs, but instead they usually stalk their prey. A typical jumping spider's predatory sequence begins when the spider detects a moving object in its neighborhood. Detection is followed by orientation towards the object and identification of such an object as prey or non-prey. If the object is identified as prey, the spider reduces the distance to it initially by a quick run and later, when close to the prey, by a slow walk and stalk. Finally the spider strikes the prey from a certain distance (Forster 1977).

During approach a stalking predator has to make significant decisions, e.g. about the direction, the speed of approach and the distance from which it can attack its prey. Different predatory decisions are associated, however, with different types of risk that may affect the outcome of the encounter (Bear & Hasson 1997). A stalking predator may fail if its prey runs or flies away even without perceiving the predator (spontaneous departure), if the prey perceives the predator and escapes before the strike (early detection), if the prey escapes during or after the strike (escape) and finally, if predatory sequence is interrupted by another predator or the spider's own enemy (interference). The analysis of all the potential risks reveals numerous trade-offs between contradictory decisions, each of which is associated with a different pay-off (Bear & Hasson 1997). For example, quick approach reduces the risk of prey's spontaneous departure and the risk of interference by other predators, but it increases the risk of predator's detection. Close approach reduces the risk of imprecise strike, but again increases the risk of predator's detection. We can assume that every factor decreasing the probability of predator's detection, such as camouflage, should change predator's decision toward the behaviors decreasing the other risks and should possibly influence predatory success. Hence, we can expect that on camouflaging background predators will attack from a shorter distance and have higher predatory success than on a non-camouflaging background. In only one study, where stalking predator's decisions were analyzed, it has been shown that a jumping spider, *Plexippus paykulli*, adapts its hunting behavior to its visibility to the prey and the ability of its prey to escape (Bear & Hasson 1997). On non-camouflaging background *P. paykulli* approached flies with higher velocities than on camouflaging background. The spider attacked the prey from longer distances on non-camouflaging than on camouflaging background. The effect of background was absent, however, when the prey were fly maggots.

The aim of this study is to check if a cryptically colored jumping spider, *Yllenus arenarius*, adapts its predatory behavior to its own visibility to the prey and to prey escape potential. This study is similar in some aspects to the study by Bear and Hasson (1997) by testing a similar problem. The study, however, uses a different model

(a highly cryptic salticid) and different prey (leafhoppers and caterpillars). Another difference is the use of living prey instead of dead prey. This enables checking how a predator's decisions affect predatory success and prey-specific behaviors, which has not been tested before.

Yllenus arenarius, a jumping spider used in this study, seems to be a particularly suitable model to test the influence of predator's visibility on its predatory decisions, because the spider is a cryptically colored stalking predator. The natural habitats of *Y. arenarius* are bare sandy areas providing very few hiding places, generally not exploited by the spiders as hunting sites. Instead, the spiders await their prey in the open, non vegetated areas, where their highly cryptic coloration provides camouflage on the sand surface (Fig. 1a). There are two major substrates occurring in the natural habitat of *Y. arenarius*: light areas of lose sand, camouflaging for juveniles, and dark patches of brown sand, which are non-camouflaging for juveniles. In this study the spiders were tested on the backgrounds possessing similar camouflaging properties to those found in the spiders' natural habitat.

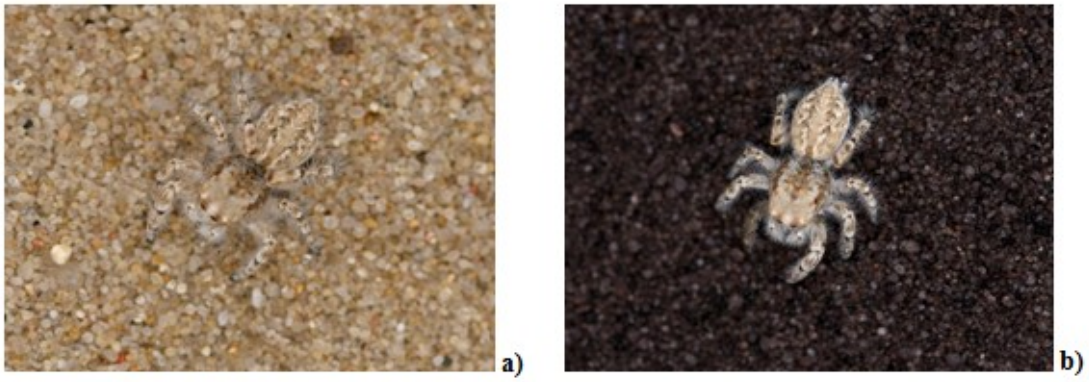

Figure 1. *Yllenus arenarius* on two backgrounds used in the experiments: a) light background, b) dark background.

Methods

The spiders used in the experiments were one-year-old juveniles of *Yllenus arenarius*. The spiders' age was determined based on their phenology, size, and maturity according to a previously developed method (Bartos 2005). All the spiders were collected from a dune in Central Poland (Kwilno, 51°59′N, 19°30′E). In order to reduce the influence of laboratory conditions on the behavior of *Y. arenarius* the experiments were carried out the same day or the day after the spiders were collected. Before the experiments the spiders were kept individually in glass containers (1000 ml) with a layer of dune sand on the bottom. After finishing the experiments the spiders were released in the field. In order to avoid using the same spiders more than once the spiders were released in the areas of the dune that were effectively isolated by dense vegetation from those areas where the spiders were later collected for the experiments.

Two insect taxa with different abilities to escape were chosen as prey animals. The leafhoppers of the genus *Arocephalus* (Hemiptera, Cicadellidae) (body length: 3.5–4 mm) were used as the prey with high escape potential. The caterpillars of *Pyralis farinalis* (Lepidoptera, Pyralidae) (body length: 6–7 mm) were used as the prey with low escape potential. Leafhoppers, including those from the genus *Arocephalus*, are common in the natural diet of *Y. arenarius* (Bartos 2011). The caterpillars of *P. farinalis* were not reported in the spider's natural diet, but the spider was found to capture the caterpillars of other lepidopteran species (Bartos 2004, 2011). The spiders were observed to use prey-specific predatory behavior against leafhoppers and caterpillars (Bartos 2007, 2008). Leafhoppers were collected in the field by sweep-netting dune grass on the day of the experiment or the day before and they were

held individually in plastic tubes. In order to reduce mortality of the prey, the insects were stored in a refrigerator at 5°C and they were taken out 15 min before the experiment started. Caterpillars were obtained from a lab culture. Each prey item was chosen randomly for the experiments.

The experiments were carried out in a white cardboard arena (15 cm high by 20 cm diameter) with a 1 cm-thick layer of sand on the bottom. Two types of backgrounds were used (Fig. 1): light background (dune sand camouflaging for the spiders), and dark background (dune sand dyed dark brown, non-camouflaging for the spiders). The sand was dyed with a brown food dye non-toxic for spiders and their prey.

Spider camouflage was judged visually. In order to reduce a possible influence of UV light, to which some insects and spiders are sensitive (Yamashita & Tateda 1976; Peaslee & Wilson 1989; Briscoe & Chittka 2001), and which is not perceived by the human eye, only artificial light sources with very low intensity of UV light (incandescent bulb) or emitting UV-C in spectra not detected by insects and jumping spiders (Li *et al*. 2008) (fluorescent tube ceiling lights emitting UV waves around 254 nm) were used in the lab. Because the spiders were tested on highly contrasting or matching backgrounds illuminated with high intensity of visible light it appears unlikely that such low intensity of UV light produced by the light sources could have a significant effect on the overall visibility of the tested spiders.

Each spider was chosen randomly for the tests and it was used only once in the whole set of experiments. The spider was first dropped onto the sand and after ten seconds a prey item was introduced about eight cm from the spider. The prey and the spider were dropped through non-transparent plastic tubes. The tube used to drop the prey was removed only when the prey stopped moving and remained motionless for 10 sec. The prey was left with the spider for 5 min and their interactions were recorded with a camera placed above the arena. In order to exclude a possibility that the spiders' reactions resulted from the activity patterns of their prey on different backgrounds all the instances when the prey moved during the spider's approach were excluded from the analysis. The fraction of excluded data was 25% or less and it was similar irrespective of the background. From the tests with leafhoppers on light background 6 of 24 trials were excluded and on dark background 5 of 23 were excluded. Sand surface was brushed between the tests to remove draglines and after that the surface layer (about 5 mm-thick) was removed. The arena was then refilled with new sand up to the previous level. All the experiments were carried out between 09:00 and 16:00 (laboratory light regime, 12L:12D, lights on at 08:00). Lighting was from a 100 W PILA incandescent bulb positioned 0.5 m above the arena and by fluorescent tube ceiling lights 2 m above the arena.

In each encounter the spider's predatory success was recorded and the jumping distance was measured. The distance was measured in Corel Draw 9.0 with a millimeter scale recorded together with the hunting sequence. Measurements were made in screen captures. The occurrence of prey-specific predatory behaviors: stalk, frontal approach and jump away (Bartos 2007), was also recorded.

The influence of background color was tested independently in approach to leafhoppers and in approach to caterpillars. Jumping distance was tested with t-test (t_n) and differences in the frequencies of prey-specific behaviors were tested with G-test ($G_{df;n}$). All analyses were performed using STATISTICA 10.0 (Statsoft, Tulsa, OK, USA) software. Statistical procedures followed those described by Zar (1984).

Results

Jumping distance was influenced by background color only if the spiders approached leafhoppers (t_{45}=6.79, p<0.001) but not if they approached caterpillars (t_{37}=1.11, p=0.27) (Fig. 2). Leafhoppers were approached and attacked from about twice

shorter the distance on light background than on dark background, while caterpillars were approached and attacked from similar distances on both backgrounds (Fig. 2).

Background color did not influence prey-specific behavior. The effect was irrespective of prey type. In approach to leafhoppers on either background there were no differences in the frequency of stalk ($G_{1;47}$=1.11, p=0.27). The spiders did not approach leafhoppers frontally and they did not temporarily release them after fang-piercing, therefore frontal approach and jump away were not recorded in the experiments with leafhoppers. Caterpillars were similarly approached and captured on light background and on dark background. Stalk was rare and occurred in similar frequencies on both backgrounds ($G_{1;39}$=0.001, p=0.97). Similar frequencies of frontal approach ($G_{1;39}$=0.13, p=0.71) and jump away ($G_{1;39}$=0.68, p=0.41) were observed on both backgrounds.

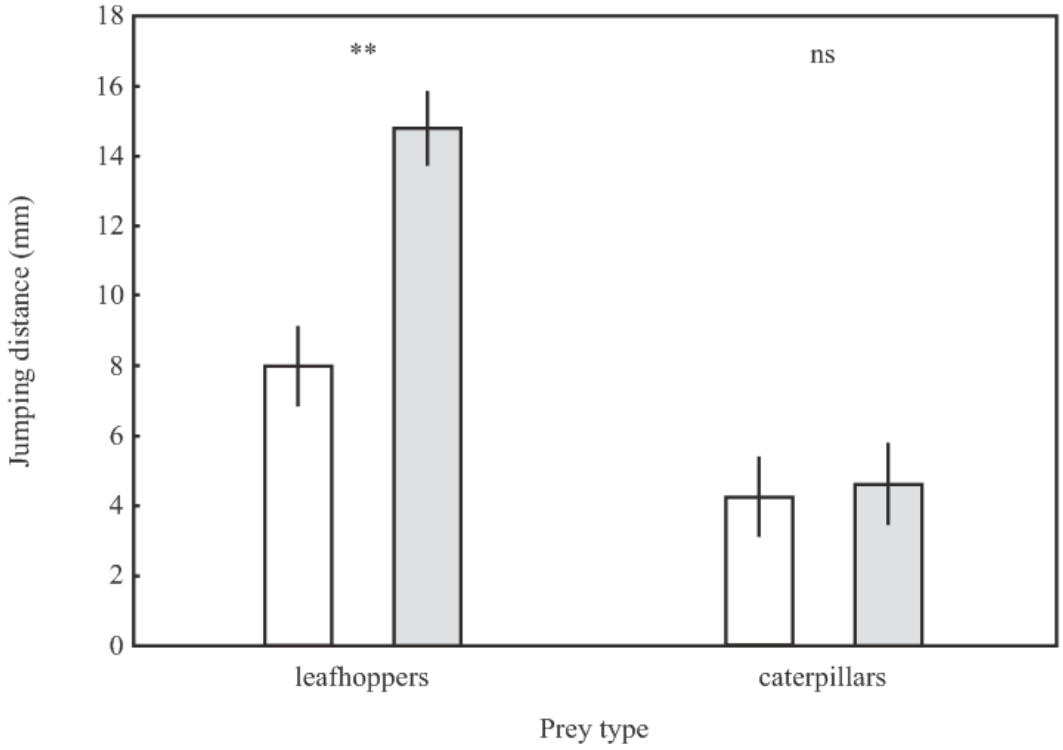

Figure 2. Jumping distance of *Y. arenarius* on leafhoppers and caterpillars on light background (white bars) and dark background (grey bars). Bars are means; whiskers are ±1.96SE; double asterisk (**), p<0.001; ns, lack of significant differences.

The predatory success of the spiders hunting leafhoppers on light background was significantly higher than on dark background ($G_{1;47}$=4.53, p=0.03). The spiders captured about 88% of leafhoppers on light background and about 61% of leafhoppers on dark background. All the prey that escaped did so after initial contact with the spider on the substrate, either during the initial strike or later, when the spider tried to subdue the prey. Predatory success of the spiders hunting caterpillars was 100% on both backgrounds, as the spiders always completed the strike and fang-pierced the caterpillars (Fig. 3).

Discussion

The results of the tests provide evidence that *Y. arenarius* adapts its predatory behavior to prey type with respect to its own visibility to the prey. The change in the behavior occurred only with leafhoppers, the prey which can escape when it detects a predator, but the spiders did not change their behavior if approaching caterpillars, the prey that

cannot efficiently escape. *Y. arenarius* and other jumping spiders were already known to use different prey-specific tactics against different prey. Alternative predatory tactics were commonly reported against the prey with high vs. low escape potential, such as flies, leafhoppers, grasshoppers possessing wings or jumping legs vs. insect larvae lacking such structures and the ability to escape efficiently (Edwards & Jackson 1993; Bartos 2007). However, the situation when a jumping spider modifies its predatory behavior in response to an environmental factor only with some prey, but not the other prey, is rare and seems to be an example of an appreciable predatory complexity and behavioral plasticity rather unusual in invertebrates. It requires from the spider to visually detect the differences in background color, and to assess the potential of the observed prey to escape, possibly by identifying certain prey characteristics. Based on the information acquired, a certain prey-capture technique is used.

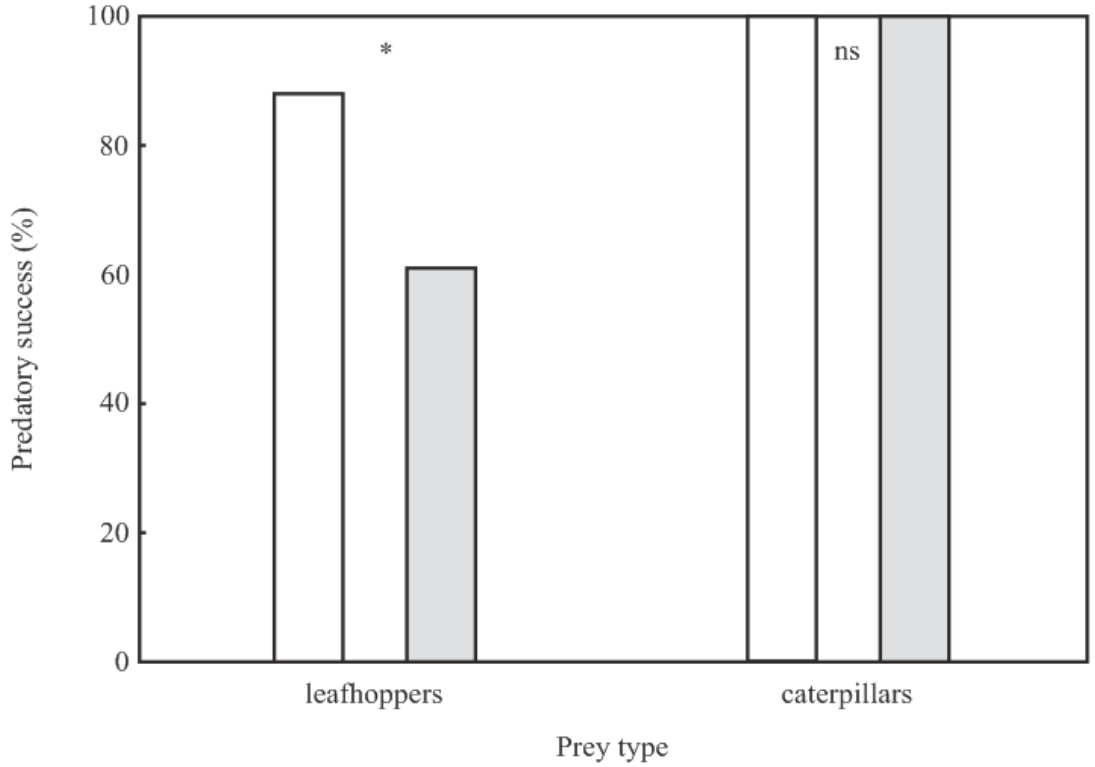

Figure 3. Predatory success of juvenile *Y. arenarius* hunting leafhoppers and caterpillars on light background (white bars) and dark background (grey bars). Asterisk (*), $p<0.03$; ns, lack of significant differences.

This is one of a very few studies showing the influence of background color on stalking predator's decisions. Another study providing similar findings was on *P. paykulli* (Bear & Hasson 1997). Prey items used in the tests with *P. paykulli* possessed similar escape abilities to those of the prey used with *Y. arenarius*. They were, however, from different insect orders. Some of the prey were anesthetized, and not live as in this study. Similar findings were also provided for several ambushing jumping spiders (Li *et al*. 2003), which suggests that the jumping spiders' predatory flexibility involving the modification of basic predatory patterns in response to different visibility to their prey may not be a rare adaptation among jumping spiders.

The differences found between the tested groups draw our attention to the trade-offs between different types of risk the predator should take into account during prey capture (Bear & Hasson 1997). *Y. arenarius* approaching leafhoppers on dark background increased jumping distance, which could reduce the risk of early detection. There was

no case of prey escape before the strike, which suggests that the risk of early detection was very low, in fact at the same level as in the case of the spiders approaching on camouflaging background. The difference in predatory success between the two groups of spiders resulted probably from the risk of failure that appears in the late phase of predation, when the attack has already been launched. The risk is related to the lower precision of attack and lower ability to subdue the prey when the attack occurs from a longer distance. This is suggested by the fact that all the attacks occurred when the prey was still on the ground and before it started to escape. Even though early detection of the predator by the prey cannot be excluded it seems a rather unlikely explanation, as all the cases in which the prey was moving before the attack were excluded from the analysis.

Interestingly, even though all the tested prey-specific behaviors could theoretically influence the outcome of predatory encounter, the differences related to background color occurred only in the jumping distance, but not the other analyzed behaviors, such as stalk, frontal approach and jump away. Stalk, the behavior specific for the tactic used against the prey with high escape potential (Edwards & Jackson 1993; Bartos 2007), seems to decrease the risk of early detection. A stalking spider moving slowly and using a characteristic choppy gait seems to reduce the risk of being noticed, at least on camouflaging background. On non-camouflaging background, however, a slowly moving spider has no concealment for a prolonged time, which should increase the risk of early detection or interference. In *P. paykulli* tested in similar conditions, stalk and other prey-specific behaviors were not analyzed, but the spider was reported to have approached faster to flies when non-camouflaged. This does not necessarily imply that the spiders stalk their prey less frequently when non-camouflaged, but may suggest some differences in predatory decisions between *P. paykulli* and *Y. arenarius*.

The other two analyzed behaviors, frontal approach and jump away, are specific for the tactic used against the prey with low escape potential. Frontal approach can generally increase the risk of early detection, but in the case of the prey that cannot escape it may have a negligible effect. In addition, frontal approach has never been observed to affect the caterpillar's velocity or the path of movement (Bartos unpubl. data). Therefore, it may not be perceived by caterpillars, even on dark background. This is especially likely for caterpillars in motion, when their own movement must notably impede the perception of the movement in their neighborhood.

The frequency of jump away analyzed in the study should, at least theoretically, affect the risk of interference by increasing the visibility of the spider and its prey. The spider hunting caterpillars usually leaves the wriggling caterpillar after initial venom injection and keeps at a distance until the venom paralyses the prey (Bartos 2007). If both the caterpillar and the spider are light in color, as in the experiment, the difference in their visibility on light vs. on dark background should result in different risks of both animals being seen on the backgrounds by a competitor or the spider's enemy. This could lead to any behaviors decreasing the risk of interference when non-camouflaged. The lack of differences between the camouflaging and the non-camouflaging background is therefore unexpected. There are no other studies to compare the results with. Bear and Hasson (1997) in their analyses had no data to discuss the risk of interference, but assumed that such a risk should occur. It seems intuitive that a predator trying to subdue a prey on a non-camouflaging background should suffer a higher risk of being noticed by its enemy than hunting on camouflaging background. The risk may, however, primarily depend on their major enemies, particularly their methods of searching the prey, sensory abilities to detect the prey and the intensity of their pressure. The majority of bare areas of sandy habitats *Y. arearius* dwells in are lacking day-active vertebrate predators or other predators with good eyesight. Major predators for the spiders

are their conspecifics, ant-lions and several ant species, other enemies, such as tiger beetles and robber flies are rather infrequent. Long-term field observations carried out for over a decade (Bartos unpubl. data) suggest that predatory pressure is generally low in the case of *Y. arenarius*, which may, at least partially, explain the lack of differences between the frequencies of jump away on the tested backgrounds.

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